A NOTE ON THE ABSORPTION SPECTRA OF THE BLOOD OF EUDISTYLIA GIGANTEA AND OF THE PIGMENT IN THE RED CORPUSCLES OF CUCUMARIA MINIATA AND MOLPADIA INTERMEDIA

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In 1868 Lankester noted the presence of a red pigment (erythrocruorin) in the plasma of certain annelids, in Chironomus larvae and in Planorbis. He also reported a green pigment (chlorocruorin) in the plasma of Siphonostoma and Sabella. Subsequent studies have revealed the widespread occurrence of erythrocruorin[2] (invertebrate hemoglobin) in many worms, in echinoderms (holothuroids), in some molluscs and in a few arthropods (Redfield, 1933; Kobayashi, 1936).[3] Chlorocruorin appears to be more limited in its distribution having been found thus far only in certain polychaete worms (Fox, 1925).

The absorption curves of both these heme pigments have been studied in some detail by Fox (1925), Redfield and Florkin (1931) and Kobayashi (1932, 1935, 1936). From a review of this literature it appears that the α-band of oxyerythrocruorin falls, in most cases, well within the 577–579 mμ region, although figures as low as 574.5 mμ have been reported for some of the worms (Barcroft and Barcroft, 1924; Kobayashi, 1936). The usual position of the α-band agrees well with the position of the comparable band in vertebrate blood. The β-band of oxyerythrocruorin occurs, in most cases, between 540–542 mμ although, here again, there are exceptions as in the case of the holothuroid, *Caudina chilensis,* where this band is found at 544.2 mμ (Kobayashi, 1932) and in the case of the earthworms, *Pheretima communissima* and *Pheretima hilgendorfi,* where the band occurs at 538 mμ and 539 mμ respectively (Kobayashi, 1936). The usual position of the β-band of oxyerythrocruorin agrees generally with the position of the comparable band in vertebrate blood. Reduced erythrocruorin, in many cases, possesses a single band with maximum at 556 mμ, which is also the case for vertebrate hemoglobin, but in *Caudina chilensis* this maximum has been reported to occur at 560 mμ (Kobayashi, 1932) and in *Cucumaria frauenfeldi* at 558 mμ (Hogben and Van Der Lingen, 1938). The situation appears to be distinctly different for some of the worms where the reduced pigment possesses a double peak; one band being at 566–571 mμ, the other at 549–551 mμ (Kobayashi, 1936; Vlès, cited by Kobayashi, 1936).

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[2] Many authors, since the original work of Lankester, have used the term hemoglobin for the pigment in invertebrates, but the present writer is following the suggestion of Svedberg and Eriksson (1933) that, since the protein portion of invertebrate hemoglobin is characteristically different from that of vertebrate hemoglobin, the separate name, erythrocruorin, is justified.

[3] A report has been published by Sato and Tamiya (1937) indicating bands of hemoglobin in *Paramecium caudatum*, but the writer has seen only an abstract of this report.

Compared to oxyerythrocruorin, the bands of oxychlorocruorin are shifted toward the red end of the spectrum. In five species of worms, Fox (1925) obtained a spectrum with the α-band in the 602.5–605.9 mμ region, while the β-band (in Spirographis) is at 561 mμ and a third faint band (also in Spirographis) is located at about 517 mμ. Using crystalline chlorocruorin, Roche and Fox (1933) have been able to confirm these results.

Recently the author had occasion to examine spectroscopically the green pigment dissolved in the plasma of the tube worm, *Eudistylia gigantea,* and the red pigment of the corpuscles found in the perivisceral fluid of the holothuroids, *Cucumaria miniata* and *Molpadia intermedia.* The results which were obtained agree in many details with previous data, but in some respects the results are so strikingly different that a record of them would be of value, even though the war has prevented completion of the investigation.

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Materials and Methods

The large tube worm *Eudistylia gigantea* (family Sabellidae) is found at relatively low tidal levels in large colonies attached to rocks or pilings in certain areas of Puget Sound. The pigment, which is dissolved in the plasma, appears red in the concentration occurring in the dorsal blood vessel, but upon removal and dilution it is seen to be green. About 0.1–0.2 ml. of blood was obtained by means of a glass capillary tube inserted into the dorsal blood vessel. After dilution to about 50–100 ml. with either cold distilled water or with cold sea water, the solution was filtered and the filtrate, containing the pigment, was placed in a 10 cm. long specimen tube. The spectrum was then obtained by visual matching, using a Bausch and Lomb spectrophotometer. The holothuroids which were used include the sea cucumber, *Cucumaria miniata* (order Dendrochirota), which is found abundantly at Friday Harbor in between rocks at low tide level, and the apodous sea cucumber, *Molpadia intermedia* (order Molpadiida), which was obtained from the muddy bottom of East Sound at a depth of 12–15 fathoms. The red pigment is located in numerous corpuscles suspended in the perivisceral fluid of both these sea cucumbers. These corpuscles in Molpadia, when examined microscopically, are seen to be pale-yellow cells which possess a variety of elongated, multi-lobed shapes. A few are oval or spherical in form and all of them have a single, small, dark and spherical nucleus located at a variety of points in the cell but rarely at the geometric center. When a drop of distilled water is mixed with a drop of the perivisceral fluid the cells assume a perfectly spherical form and the nucleus is seen to occupy an eccentric position. The corpuscles of Cucumaria are also pale-yellow, nucleated cells with a variety of shapes; some are ovoid, some spherical and others are quite irregular with one or several processes. The elongated lobed forms of Molpadia are seldom seen in *Cucumaria miniata.* Previous accounts of the holothurian red cell by Dawson (1933) and Ohuye (1936) have already mentioned some of these structural features. By means of a small puncture in the body wall, the red perivisceral fluid was collected and the cells centrifuged out. After decanting the supernatant fluid, the cells were washed in cold sea water and again centrifuged out. This process was repeated three or four times after which the cells were hemolyzed in cold distilled water. The solution of pigment after filtration and dilution to about one hundred ml. with cold distilled water was examined spectroscopically.

Results and discussion

The five curves (Fig. 1) obtained from five different sea cucumbers (*Cucumaria miniata*) give an indication of the reproducibility of the spectral curve and show that the location of a point of maximum or minimum can be checked within 2 mμ. These curves, which were obtained from well-aerated solutions of the pigment, show two maxima; one 580–581 mμ, the other at 544–545 mμ. The point

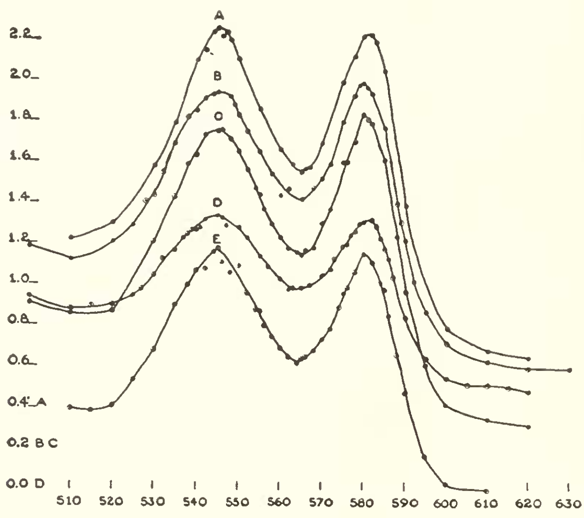

FIGURE 1. The absorption spectral curves of the pigment from five different sea cucumbers (*Cucumaria miniata*). The density (log I_0/I) is plotted as ordinates against the wave length, in mμ. The individual graphs are plotted on a multiple ordinate scale to avoid crowding. The zero ordinate for each curve is indicated by the corresponding letter. Each plotted point represents the mean of 3–5 spectrometric readings.

of minimum absorption between these two maxima is at 564–565 mμ. A comparison of the spectral curves of the pigments, in the oxidized state, from all three species is shown in Figure 2. It is evident that all three pigments show two maxima, but the maxima for Eudistylia and for Molpadia are shifted toward the red end of the spectrum when compared with the Cucumaria data, the shift being greater in the case of Molpadia. The position of the maximum and minimum points for all the curves that were obtained from all three organisms are listed in Table I.

Reduction of the pigment by means of sodium hyposulphite results in a radical, though reversible, change in the spectral curve for all three pigments (Fig. 3). In the case of Cucumaria the original bands disappear and two new bands, one at 562–563 mμ, the other at 530–532 mμ, make their appearance. The point of minimum between these two maxima is located at 545 mμ (Table I). In Eudistylia, reduction results in the disappearance of the two original bands and in the appearance of a band with a peak at 577–580 mμ. There is also an indication of a secondary band at about 540 mμ (Fig. 3) but the data on this point are too meagre to merit any degree of confidence. Reduction of the pigment from Molpadia, again with hyposulphite, leads to the replacement of the two original bands by a single band at 588 mμ. It is possible that, here too, more data would reveal the existence of a secondary band since an indication of this is visible in the curve for Molpadia

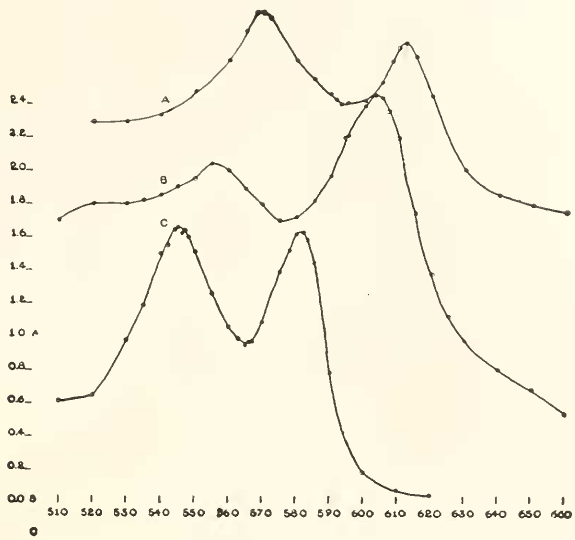

FIGURE 2. The absorption curves of the oxidized pigment from *Molpadia intermedia* (*A*), *Eudistylia gigantea* (*B*), and *Cucumaria miniata* (*C*). The curves are plotted on a multiple ordinate scale. Other details as Figure 1.

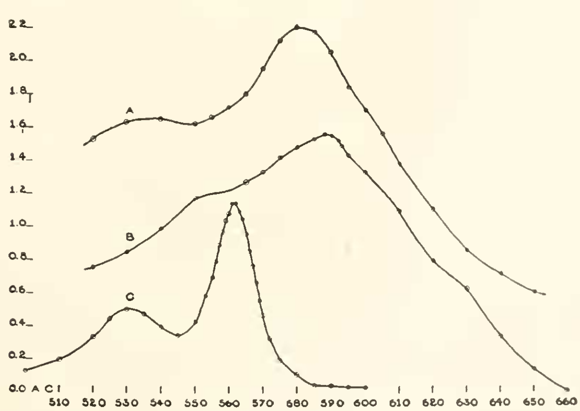

FIGURE 3. The absorption curves of the pigment after reduction with sodium hyposulphite from *Eudistylia gigantea* (*A*), *Molpadia intermedia* (*B*), and *Cucumeria miniata* (C). The curves are plotted on a multiple ordinate scale. All other details as in Figure 1.

TABLE I

Species *		Oxidized state			Reduced state	
		α	β	Minimum *	Main	Secondary
Caudina chilensis		579.5	544.2	564.5	560	
Molpadia roretzii		577	541.5	562	557	
Cucumaria frauenfeldi		579	543		558	
Spirographis spallanzi (crystals)		602.5	562.5			
Eudistylia gigantea	1	605	555	576	580	540
	2	604	555	576	577	
	3	603	558	577		
	4	602	554	575		
Cucumaria miniata	1	581	545	564	562	
	2	580	545	564	561.5	530
	3	580	544	564	562	531
	4	580	544	565	562	531
	5	580	547	565	562	531
	6	582	545	565	563	532
	7				563	535
Molpadia intermedia	1	615	570	592	588	
	2	612	570	592	588	
	3	611	568	595		

* The position of the minimum between the α- and β-bands.
The data from Caudina and *Molpadia roretzii* were taken from Kobayashi (1932).
The data for *Cucumaria frauenfeldi* were taken from Hogben and Van Der Lingen (1928).
The data for Spirographis were taken from Roche and Fox (1933).

(Fig. 3). It is clear, then, that reduction of the pigment from all three organisms leads to a hypsochromic shift, but the shift is not one of equal magnitude for all three organisms, since the span from the α-band to the principal band of the reduced pigment is about 18 mμ for Cucumaria, 25 mμ for Molpadia and 26 mμ for Eudistylia.

It seems very likely that the pigment in the red cells of *Cucumaria miniata* is erythrocruorin. The positions of the α- and β-bands, as well as the point of minimum, agree reasonably well with the previous data obtained with other holothurians (Table I). The reduced pigment from Cucumaria shows two bands; the main band at 562–563 mμ agrees approximately with the 557–560 mμ band previously reported for holothurians (Table I), but the secondary band at 530–532 mμ is previously unreported, although from the plasma pigment of certain worms (Kobayashi, 1936) a spectral curve has been obtained which has a 2-banded structure with the secondary band in the 549–551 mμ region.

It also seems likely that the green pigment dissolved in the plasma of *Eudistylia gigantea* is chlorocruorin. The α-band at 602–605 mμ (Table I) agrees completely with the data given by Fox (1925) and by Roche and Fox (1933), although the present data shows the β-band to be shifted toward the violet end of the spectrum by about 6–7 mμ as compared with the position of the β-band given by the abovementioned workers. The principal band of the reduced chlorocruorin from Eudistylia has a peak at 577–580 mμ whereas the data of Fox (1925) for Spirographis place it at about 574 mμ.

The results obtained from *Molpadia intermedia* are surprising. At the outset there was no reason to suspect that an absorption curve agreeing approximately with that obtained by Kobayashi (1932) from *Molpadia roretzii,* and indicating an erythrocruorin, should not be obtained. Instead, as Figures 2 and 3 indicate, the bands for both the oxidized and reduced pigment are shifted toward the red end to a degree even greater than in the case of chlorocruorin. The α-band of *Molpadia intermedia* occurs 36 mμ further toward the red than the corresponding band for *Molpadia roretzii,* while the band of the reduced pigment of *Molpadia intermedia* is 31 mμ further toward the red than in *Molpadia roretzii.* It is also significant to note that the span between the α- and β-bands in other sea cucumbers (Table I) is 35–36 mμ whereas the corresponding span in *Molpadia intermedia* is about 43 mμ. These differences appear to be too great to be accountable to errors in measurement or to the usual species differences that are known to occur. The spectrum of the pigment from *Molpadia intermedia* does not agree with the spectrum of hemerythrin, the red pigment found in certain Gephyrean worms as well as in the polychaete, Magelona (Marrian, 1927). It must be concluded that either this represents the true absorption spectrum of a heme pigment (if it is a heme pigment) characteristically different from either erythrocruorin or chlorocruorin, or that unrecognized conditions cause a shift of the bands from the typical positions of erythrocruorin. The onset of war resulted in the sudden interruption of the investigation at this point so that this final question must remain unanswered till a later date. A thorough examination of the crystallized pigment by chemical as well as by spectroscopic means should be made before the existence of a new pigment can be accepted.

Summary

A spectrometric examination of the green pigment dissolved in the plasma of the tube worm, *Eudistylia gigantea,* and of the red pigment in the corpuscles of the sea cucumbers, *Cucumaria miniata* and *Molpadia intermedia,* has led to the following conclusions.

1. The green pigment of Eudistylia appears to be chlorocruorin. In the oxidized state it possesses an α-band at 602–605 mμ and a β-band at 554–555 mμ. In the reduced condition a main band occurs at 577–580 mμ with a second band suggested at 540 mμ.

2. The red pigment of Cucumaria appears to be erythrocruorin. In the oxidized state an α-band at 580–581 mμ and a β-band at 544–545 mμ are seen. When the pigment is reduced a band appears at 562–563 mμ as well as one at 530–532 mμ.

3. The red pigment of Molpadia, when oxidized, possesses a band at 611–615 mμ and another at 568–570 mμ. In the reduced condition a band at 588 mμ is evident. This spectrum does not agree with the spectrum of either chlorocruorin or of erythrocruorin. The new spectrum may indicate the existence of another pigment with the ability to combine reversibly with oxygen.

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